Eur J Plant Pathol (2013) 135:187–200
DOI 10.1007/s10658-012-0077-3

Effects of sharp eyespot (*Rhizoctonia cerealis*) on yield and grain quality of winter wheat

Grzegorz Lemańczyk · Hanna Kwaśna

Accepted: 22 August 2012 / Published online: 7 October 2012
© The Author(s) 2012. This article is published with open access at Springerlink.com

Abstract Sharp eyespot caused by *Rhizoctonia cerealis* was assessed in four winter wheat crops surveyed at four locations in north-central Poland in 2006–2009. At the four locations symptoms developed on 41.9–67.7 % of shoots of all plants (average of 4 years) and on 49–73.5 % of shoots of diseased plants (average of 4 years). Slight (category 1) disease was most frequent, occurring on 24.4–41.3 % of shoots (range 14.8–51.3 %); moderate (category 2) disease was less frequent, occurring on 16.9–25.5 % of shoots (range 8.9–32.4 %); severe (category 3) disease was least frequent, occurring on 3.2–7.1 % (range 0–22.0 %) of shoots. Sharp eyespot affected wheat growth and yield, and grain quality. Disease, especially in the severe category, was associated with significant decreases in plant and ear dry weights, number of grains per ear, grain dry weight per ear and thousand-grain weight, and with increases in grain protein and wet gluten contents, Hagberg falling number and sedimentation value. There was an association between occurrence of sharp eyespot in stems and colonization of grain by fungi. *Alternaria alternata* and *E. nigrum* were the most common species.

Keywords Grain quality · *Rhizoctonia cerealis* · Sharp eyespot · Wheat

G. Lemańczyk
Department of Phytopathology and Molecular Mycology,
University of Technology and Life Sciences,
Kordeckiego 20,
85-225 Bydgoszcz, Poland

H. Kwaśna (✉)
Department of Forest Pathology,
Poznań University of Life Sciences,
Wojska Polskiego 71c,
60-625 Poznań, Poland
e-mail: kwasna@up.poznan.pl

Introduction

In 2009 the world production of wheat (*Triticum aestivum* L.) was 682 million tonnes, making it the second most-produced cereal after maize (Anonymous 2010). In the human diet, wheat is the largest source of vegetable protein and provides also carbohydrates, minerals and vitamins (Anonymous 2006). The wheat grain used in human diet has to be of sufficient quality for consistent technological processing and good quality of bakery and other products. The wheat protein, gluten, determines the suitability of grain for baking.

Fungal infection and presence of diseases on wheat plants may affect quantity and quality of grain (Prange et al. 2005; Siuda et al. 2010).

Sharp eyespot caused by the soil-borne fungus *Rhizoctonia cerealis* van der Hoeven (teleomorph: *Ceratobasidium cereale* D. Murray & L.L. Burpee) is a stem-base disease of wheat. The disease is also common among other plants of *Poaceae* including barley, oats and rye.

Rhizoctonia cerealis is present throughout the temperate wheat growing regions of the world. The fungus

over-winters as mycelium or sclerotia in plant debris or in the soil. Mycelium from infected plant debris or from germinating sclerotia serves as primary inoculum. The fungus does not form asexual spores and the sexual stage is rare in nature. Infection of plants occurs at the root tissues at any time during the growing season. The symptoms in wheat include dark-bordered lesions on stem bases of young and mature plants. Lesions may coalesce into large patches girdling the stem. Sclerotia may be formed between the lower leaf sheath and stem or within the stem lumen of severely infected shoots. The fungus may cause pre- and post-emergence damping off and shoot death of seedlings (Willis 1984; Wiese 1987; Nyvall 1989; Carling and Sumner 1992). Slight incidence of sharp eyespot tends to have little effect on yield. Severe infection of mature shoots may result in small, shrivelled grain and can induce lodging at the second or third internode and cause premature spike senescence or ripening (white heads). Exceptionally, shoots may be killed as the ear emerges from the sheath (Clarkson and Cook 1983; Cromey et al. 2002). The disease is favoured by neutral to slightly acid, dry and sandy soils (Pitt 1964). Cool autumn or spring may result in earlier infection and more severe attacks. Disease incidence tends to be greater in continuously cropped cereals. Practices that help reduce the effects of the disease include crop rotation and late sowing in autumn.

The incidence of sharp eyespot in Europe is increasing (Rossi et al. 1995; Mikolajska and Wachowska 1996; Colbach et al. 1997; Kryuchkova 2000; Tunali et al. 2008; Zhalieva 2008). The increase has been related to:

1. earlier sowing (Colbach et al. 1997);
2. increased use of fungicides, especially those for controlling fusarium root rot (*Fusarium* spp.) and eyespot (*Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams and *O. acuformis* (Boerema, R. Pieters & Hamers) Crous & W. Gams), which decrease microbial antagonism to favour *R. cerealis* (Prew and McIntosh 1975; van der Hoeven and Bollen 1980);
3. increased geographic distribution of *R. cerealis* and its possible spread to new areas;
4. possible increase in pathogen's aggressiveness after transfer from natural plant communities to agricultural crops (Chakraborty et al. 2000);
5. wider distribution of wheat cultivars susceptible to *R. cerealis* (Cromey et al. 2005);
6. more favourable weather for infection, particularly a combination of a longer, colder pre-winter growing period and wet autumn and spring (Clarkson and Cook 1983).

Formal surveys of sharp eyespot incidence in Poland have not been made. The disease has, however, been observed regularly in recent years (Lemańczyk unpublished), prompting an evaluation of its potential economic effects.

In the present study we determined the incidence and severity of sharp eyespot on wheat crops at four locations in Poland in four growing seasons, 2006–2009, and investigated its effects on grain yield and grain quality.

Materials and methods

Survey

Four commercial crops of winter wheat were grown in successive years (harvested in 2006–2009) at each of four sites, Chrząstowo, Minikowo, Mochełek and Sobiejuchy, located 20–70 km apart, in north-central Poland (Kuyavia-Pomerania) (Fig. 1). A different cultivar was grown at each site (Table 1). Each crop was grown as a first wheat, after winter oilseed rape, or as a second wheat (Mochełek in 2007 and 2008). The weather data, type of soil and management procedures for each crop are presented in Table 1.

Incidence and severity of sharp eyespot were determined at growth stages (GS) 13–14, 30–31, 43–45 and 87, and grain yield and quality were determined at GS 87 (Zadoks et al. 1974) to avoid possible failure in the event of further disease development. The Minikowo site was not sampled in 2008. All plants from four plots (1 m×1 m each), located at similar intervals along a diagonal transect across the field, were harvested by hand. Plants were separated into three groups: those with symptoms of sharp eyespot, those with symptoms of other diseases and those with no symptoms of disease (control).

Sharp eyespot assessment

Sharp eyespot severity was assessed on plants with symptoms of disease, on leaf sheaths and stems of each main shoot and tiller, according to the following

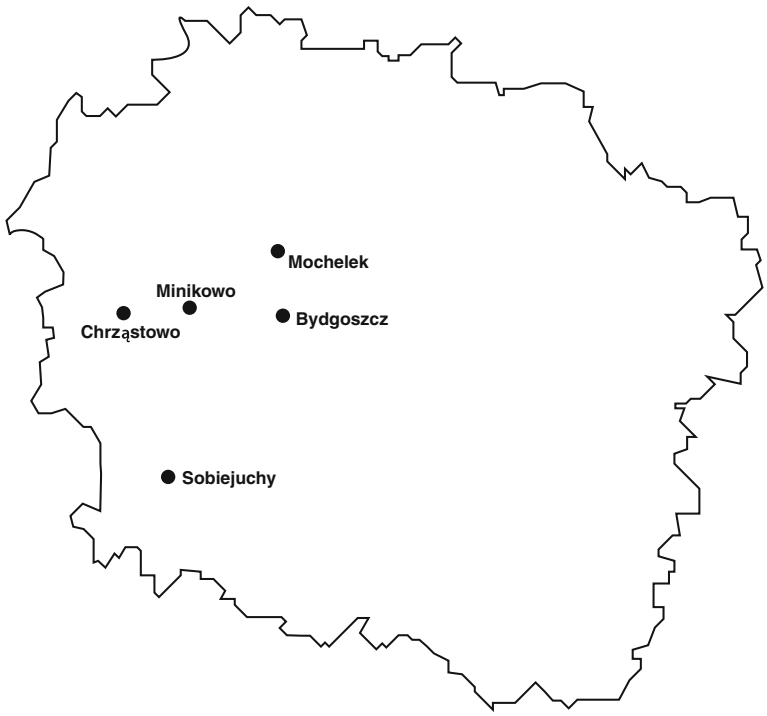

Fig. 1 Location of surveyed fields in the Kuyavia-Pomerania Voivodeship

key: 0—healthy, no symptoms of sharp eyespot; 1—slight disease, one or more lesions on stem girdling in total less than half the stem circumference or on leaf sheath, not penetrating to the stem; 2—moderate disease, one or more lesions on stem, girdling in total at least half the stem circumference; 3—severe infection, one or more lesions on stem, girdling in total more than half the stem circumference, stem weakened (Clarkson and Cook 1983).

Detection of *Rhizoctonia cerealis*

The presence of *R. cerealis* was confirmed by morphology and molecular analysis. Cultures of fungus grown from infected wheat tissues after their surface-disinfection in silver nitrate (1 % $AgNO_3$) for 20 s, rinsing in sterile distilled water, sectioning into 1-cm pieces, placing on potato dextrose agar (PDA, Difco + 50 mgl^{-1} streptomycin) and incubation for 14 days at 22 °C, were examined microscopically. Molecular analysis was done after direct extraction of DNA from visibly infected leaf sheaths and stem bases. Primer set Rc2F/R (Nicholson and Parry 1996) was used to establish the presence of *R. cerealis.*

Pathogenicity tests

Mycelium of *R. cerealis* grown on PDA was used to inoculate grain or climate chamber-grown plants. In both tests winter wheat cv. Tonacja was used. The grain was surface-disinfected in mercury (II) chloride (1 % $HgCl_2$) for 5 min, rinsed in sterile distilled water 6×10 min, placed on sterile wet blotting paper in Petri dishes and incubated at 20–22 °C for 48 h. Germinated grains were transferred to 10-mm PDA discs with 10-day-old *R. cerealis* mycelium on three sheets of sterile, wet (with 16 ml of water) blotting paper per dish (200×30 mm). Infection was assessed after 7–16 days. Each of four isolates of *R. cerealis* was tested on 80 grains in four Petri dishes.

Plants were inoculated at the beginning of stem elongation (GS 31). Discs of PDA (5 mm) with 14-day-old *R. cerealis* mycelium were applied to the stem base and leaf sheath using Parafilm. Plants were maintained in a climate chamber at 20 °C. Discs were removed after 5 days and plants observed regularly for development of symptoms.

Table 1 Crop management procedures in first wheat crops grown in 4 years at four sites

Location	Chrząstowo		Minikowo		Mochełek		Sobiejuchy	
Geographic coordinates	53°09′N 17°35′E		53°29′N 17°56′E		53°13′N 17°51′E		52°54′N 17°43′E	
	Average temp. (°C)	Precipitation (mm)	Average temp. (°C)	Precipitation (mm)	Average temp. (°C)	Precipitation (mm)	Average temp. (°C)	Precipitation (mm)
2005/2006	7.8	460	7.9	485	7.4	490	8.0	490
2006/2007	10.1	620	10.3	640	9.7	610	10.3	570
2007/2008	8.7	490	8.7	440	8.6	460	8.9	960
2008/2009	8.2	525	8.5	430	8.1	580	8.6	670
Soil	Silty loam		Loam		Sandy loam		Silty loam	
Wheat cultivar	Cubus		Trend		Tonacja		Smuga	
Preceding crop	Winter oilseed rape (*Brassica napus* L. var. *napus*)		Winter oilseed rape		Winter wheat - 2006, 2007 Winter oilseed rape - 2008, 2009		Winter oilseed rape	
Pre-preceding crop	Spring barley (*Hordeum vulgare* L.)		Spring barley		White mustard (*Sinapis alba* L.) - 2006, 2007		Triticale (× *Triticosecale* Wittm. ex A.Camus) - 2006, 2007, 2008	
					Spring barley - 2008, 2009		Spring wheat - 2009	
Level of mineral fertilization, kg·ha^{-1}	N P K		N P K		N P K		N P K	
2006	101	28 59	103	25 47	80	17 50	102	21 81
2007	106	36 54	105	25 47	80	17 50	107	23 87
2008	108	31 46	0	0 0	80	17 50	105	18 83
2009	98	24 66	108	25 47	80	17 50	105	18 83
Fungicide[a] application								
2006	GS 30–31[2]	Alert 375 SC	Charisma 207 EC		Alert 375 SC		Allegro 250 SC	
	GS 49–59	Falcon 460 EC	Amistar 250 SC		Artea 330 EC		Amistar 250 SC	
2007	GS 30–31	Allegro 250 SC	Juwel TT 483 SE		Alert 375 SC		Juwel TT 483 SE	
	GS 49–59	Falcon 460 EC	Amistar 250 SC		Artea 330 EC		Amistar 250 SC	
2008	GS 30–31	Juwel TT 483 SE	None		Alert 375 SC		Opera Max 147,5 SE	
	GS 49–59	Amistar 250 SC	None		Artea 330 EC		Swing Top 183 SC	
2009	GS 30–31	Juwel TT 483 SE	Opera Max 147,5 SE		Alert 375 SC		Wirtuoz 520 EC	
	GS 49–59	Fandango 200 EC	Fandango 200 EC		Artea 330 EC		Charisma 207 EC	
							Talius 200 EC	

[a] Fungicide active ingredients:

Alert 375 SC (125 gl^{-1} flusilazole +250 gl^{-1} carbendazim), Du Pont de Nemours (France) S.A.S

Allegro 250 SC (125 gl^{-1} kresoxim-methyl +125 gl^{-1} epoxiconazole), BASF SE Germany

Amistar 250 SC (250 gl^{-1} azoxystrobin), Syngenta Crop Protection, Switzerland

Artea 330 EC (250 gl^{-1} propiconazole +80 gl^{-1} cyproconazole), Syngenta Crop Protection, Switzerland

Charisma 207 EC (106.7 gl^{-1} flusilazole +100 gl^{-1} famoxadone), DuPont Poland Sp. Z o.o

Falcon 460 EC (250 gl^{-1} spiroxamine +167 gl^{-1} tebuconazole + 43 gl^{-1} triadimenol), Bayer CropScience AG, Germany

Fandango 200 EC (100 gl^{-1} prothioconazole +100 gl^{-1} fluoxastrobin), Bayer CropScience AG, Germany

Opera Max 147,5 SE (85 gl^{-1} pyraclostrobin +62.5 gl^{-1} epoxiconazole), BASF SE, Germany

Juwel TT 483 SE (83 gl^{-1} epoxiconazole +83 gl^{-1} kresoxim-methyl + 317 gl^{-1} fenpropimorph), BASF SE, Germany

Swing Top 183 SC (133 gl^{-1} dimoxystrobin +50 gl^{-1} epoxiconazole), BASF SE, Germany

Talius 200 EC (200 gl^{-1} proquinazid), DuPont Poland Sp. Z o.o

Wirtuoz 520 EC (320 gl^{-1} prochloraz +160 gl^{-1} tebuconazole + 40 gl^{-1} proquinazid), DuPont Poland Sp. Z o.o

[2] Fungicides were applied at the beginning of stem elongation (GS 30–31) and during heading (GS 49–59)

Measurement of wheat yield

One hundred shoots from each crop were assessed for effects of sharp eyespot severity on yield. Plant and ear dry weights and the number of grains per ear were determined. Grain from individual ears was threshed, dried and weighed. Grain was separated into fractions according to length, i.e. <2 mm, 2–2.5 mm, 2.5–2.8 mm and >2.8 mm, and percentage of grains in each size category was determined for each sharp eyespot severity category. Thousand-grain weights were determined using 400 grains taken at random from a 500 g sample from each severity category. Germination rate of grain (% seeds germinating) from each severity category was determined. The yield of grain from the sample area was determined.

Measurement of grain quality

Grain was evaluated for four technological traits: total protein and wet gluten contents, Hagberg falling number and sedimentation value. Hagberg falling number is an indicator of alpha-amylase activity in the flour. A high value indicates low alpha-amylase activity, showing that the flour is less degraded by the enzyme. Sedimentation value indicates the baking quality of flour. Higher values indicate better quality of total protein and strength of gluten. Each analysis was done twice, each time on 15–20 g of grain taken at random from each severity category.

Total protein content and sedimentation value were determined by near-infrared spectroscopy (NIR). Wet gluten content was determined by the standard procedure, PN-A-74043-2. This included preparation of dough (10 g of ground grain +5.5 ml 2 % NaCl), extraction of wet gluten from the dough (by washing it out with 2 % NaCl), removing excess water (by centrifuge) and weighing the dried mass of gluten. Hagberg falling number was determined by the standard procedure, PN-ISO-3093, which follows the ICC (International Association of Cereal Science and Technology) standard method No. 107.

Association of sharp eyespot with colonization of grain by other fungi

Colonization of grain by fungi was studied on samples taken from shoots with disease in severity categories 2 and 3, and healthy controls. One hundred grains, selected randomly from each sample, were rinsed in tap water for 45 min, surface-disinfected in sodium hypochlorite (1 % available chlorine) for 3 min, rinsed 3×10 min in sterile distilled water and placed on PDA (pH 5.5) in Petri dishes (six grains per plate). In 2007–2009 an additional 100 grains from the same disease categories were placed on PDA without rinsing or surface disinfection. After incubation for 10 days at 22 °C, all cultures were transferred to PDA slants for preservation. Representative cultures were identified according to their morphology on potato dextrose agar (PDA; 40 g filtered white potatoes, 20 g agar, 1 l distilled water, pH=7) and synthetic nutrient agar (SNA; 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 20 g agar, 10 mg chlorotetracycline, 50 mg dihydrostreptomycin sulphate, 1 l distilled water) and available literature. Some dematiaceous hyphomycetes were induced to sporulate under UV light (310–420 nm for 12 h/day) at 20 °C, or on 2 % MEA at 5 °C in high humidity, for 12–15 months. Fungi recorded from non-disinfected grains were considered to have grown, at least partly, from the surface of the grains, and those recorded from surface-disinfected grains were considered to have grown from inside of the grain. The identity of *Fusarium langsethiae* was confirmed by PCR assay with the use of SCAR (sequence characterized amplified region) primers (Wilson et al. 2004).

Statistical analyses

Data for sharp eyespot incidence and all plant growth, grain yield and grain quality measurements, log-transformed where appropriate, were subjected to one-way or three-way analysis of variance (ANOVA, Huynh and Feldt 1970). Where interactions (severity × location and severity × year) occurred, data were (i) plotted to determine the factors responsible, and (ii) subjected to Scheffé's method (Maxwell and Delaney 1990) for estimation of significance of the differences between averages. Where there were no factorial interactions, the data are discussed individually. Statistical significance was assumed at $P \leq 0.05$ or $P \leq 0.001$ using Matlab 7.3.0 with Statistical Toolbox version 5.3 (MathWorks, Inc., Natick, MA, USA). The statistical

significance of difference between numbers of fungal isolates from two different grain samples was determined by a χ^2 - test according to the formula

$$\chi^2 = (n_i - nP_i)^2 / nP_i \quad i = 1$$

where n is the number of isolates and P the probability of occurrence.

Results

Survey and sharp eyespot assessment

Sharp eyespot was recorded in crops surveyed in Chrząstowo, Minikowo, Mochełek and Sobiejuchy, in north-central Poland, in four successive years (Table 2). The progress of disease in individual sites and years, represented by the percentage of diseased stems in a sample of healthy + diseased plants, is shown in Fig. 2. In the sample that included healthy + diseased plants, average percentage of shoots in different disease categories was: (13.7−) 32.3–58.1 (−84.6) in category 0; (11.6−) 16.7–36.3 (−49.0) in category 1; (3.2−) 10.9–23.7 (−30.2) in category 2; (0−) 2.6–11.5 (−35.6) in category 3. In the sample that included only diseased plants, symptoms developed on 49–73.5 % of shoots. Average percentage of shoots infected was: (12.2−) 26.5–51.0 (−70.4) in category 0; (14.8−) 24.4–41.3 (−51.3) in category 1; (8.9−) 16.9–25.5 (−32.4) in category 2; (0) 3.2–7.1 (−22.0) in category 3 (Table 2). The

Table 2 Percentage of shoots with sharp eyespot in different severity categories in healthy + diseased plants (A) and diseased plants (B) and the yield of grain from the sample area

	No. of stems examined		Severity category								Yield of grain (t·ha^{-1})
			0		1		2		3		
	A	B	A	B	A	B	A	B	A	B	B
Chrząstowo											
2006	774	696	64.3	70.4	18.3	14.8	17.4	14.8	0.0	0.0	6.81
2007	821	581	17.2	12.2	24.8	34.6	22.4	31.2	35.6	22.0	6.98
2008	1089	314	84.6	57.4	12.2	33.4	3.2	9.2	0.0	0.0	7.96
2009	809	609	66.4	63.8	11.6	14.9	11.6	14.9	10.4	6.4	8.15
Average in 2006–2009	873	550	58.1	51.0	16.7	24.4	13.7	17.5	11.5	7.1	7.48
Minikowo											
2006	760	715	63.1	63.7	18.9	19.2	18.0	17.1	0.0	0.0	6.81
2007	900	474	58.0	39.0	18.0	32.5	16.2	19.0	7.8	9.5	6.94
2009	784	443	53.0	44.2	36.9	41.1	10.1	14.7	0.0	0.0	7.93
Average in 2006–2009	815	544	58.0	49.0	24.6	30.9	14.8	16.9	2.6	3.2	7.23
Mochełek											
2006	800	554	56.9	44.4	25.9	33.0	12.8	19.0	4.4	3.6	4.17
2007	722	643	28.2	28.2	47.9	51.3	14.3	13.7	9.6	6.8	3.86
2008	860	485	61.1	49.5	30.3	37.1	5.2	8.9	3.4	4.5	3.81
2009	1028	509	54.3	31.4	16.6	31.4	11.4	28.9	17.7	8.3	3.94
Average in 2006–2009	853	548	50.1	38.4	30.2	38.2	10.9	17.6	8.8	5.8	3.95
Sobiejuchy											
2006	791	336	63.0	39.5	18.0	41.7	10.0	12.5	9.0	6.3	7.85
2007	795	712	28.7	30.3	36.4	37.4	26.7	26.7	8.2	5.6	7.99
2008	764	616	13.7	16.4	49.0	44.2	30.2	32.4	7.1	7.0	8.11
2009	792	504	23.9	19.8	42.0	41.9	27.8	30.4	6.3	7.9	8.32
Average in 2006–2009	786	542	32.3	26.5	36.3	41.3	23.7	25.5	7.7	6.7	8.08
Average of all locations and years			49.6	41.2	27.0	33.7	15.8	19.4	7.7	5.7	6.68

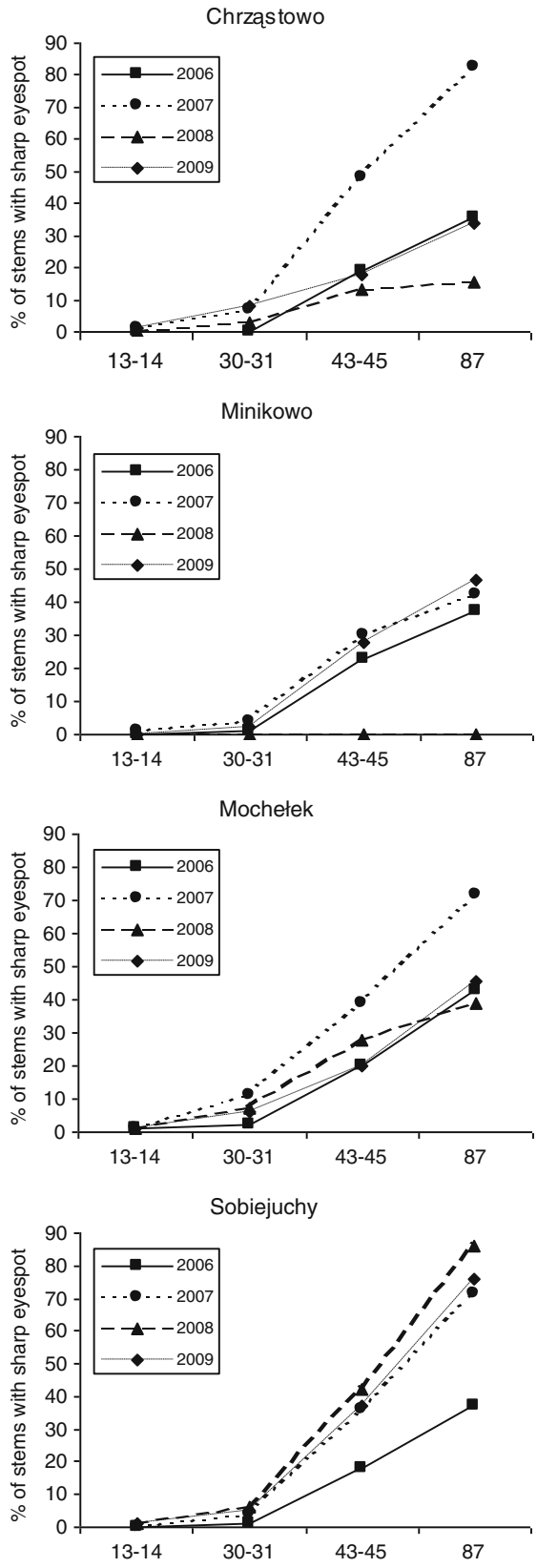

Fig. 2 Incidence of the sharp eyespot in Chrząstowo, Minikowo, Mochełek and Sobiejuchy in 2006–2009

proportions of main shoots and secondary tillers infected were similar. The highest proportions of shoots infected were in the least severe disease categories. The most severe disease occurred at three sites in 2007 and was associated with highest average temperature and total precipitation (Fig. 2, Table 1). The higher average temperature in 2006/2007 resulted from a relatively warm and wet autumn and winter and following spring, but relatively cool and wet summer. The generally greater amount of disease at Sobiejuchy in 2006–2008 was associated with most grain yield (8.08 tha^{-1}). Mixed symptoms of sharp eyespot and other diseases such as true eyespot were found rarely.

Detection of *Rhizoctonia cerealis*

Growth rate in vitro and morphology of mycelium isolated from lesions, identified as sharp eyespot, conformed to the original description of *R. cerealis*. The taxonomy of *R. cerealis* was confirmed additionally with PCR. The specific *R. cerealis* primer, SCAR Rc2 F/R type, amplified DNA from wheat leaf sheath and stem base samples and from the isolated mycelium giving a DNA product of 800-bp. Koch's postulates were satisfied by re-infection and re-isolation of the pathogen. In pathogenicity tests, 7–16 days after re-infection, most isolates of *R. cerealis* produced lesions typical of sharp eyespot on the wheat coleoptiles, stems, leaves and sometimes on roots. Stem infection caused the plants to die eventually. Mycelium was isolated from the leading edge of developing rachis lesions. Its colony morphology and hyphal branching were consistent with the applied *R. cerealis.*

Grain yield and quality

Increasing severity of sharp eyespot generally decreased plant and ear dry weights, number of grains per ear, grain dry weight per ear and thousand-grain weight (Table 3). Greatest effects were usually observed at Minikowo and Mochełek. Grain variates were often affected, with statistical significance, by sharp eyespot severity category (0–3), location (Ch, Mi, Mo, So), and year (2006, 2007, 2008, 2009) (Table 4). Factorial interactions, severity × location and severity × year, were also sometimes significant.

Table 3 Effects of sharp eyespot severity category on wheat growth, grain yield, grain germination and quality in 2006–2009 (averages)

	Chrząstowo				Minikowo				Mochełek				Sobiejuchy			
	Severity category															
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	
Plant dry weight (g)	3.86	3.55	3.30*	3.65	3.63	3.27	2.99*	2.49**	3.07	2.80	2.48*	2.34*	3.57	3.34	2.91*	2.33**
Ear dry weight (g)	2.46	2.24	2.08	2.28	2.32	2.06	1.85*	1.56**	1.77	1.64	1.41*	1.24*	2.21	2.03	1.75*	1.36**
Number of grains per ear	51.1	48.4	44.9*	50.1	48.6	42.7*	42.0*	47.3	35.6	33.7	30.9	29.2*	40.3	38.5	35.2	30.1**
Grain dry weight per ear (g)	2.00	1.82	1.66	1.89	1.89	1.60	1.50*	1.41*	1.45	1.34	1.14*	0.96*	1.79	1.70	1.48	1.16**
Grain fraction <2 mm (%)	1.0	0.9	1.0	1.25	0.8	1.6	1.4	3.4	0.7	0.6	1.4	2.8	0.3	0.3	0.8	1.4
Grain fraction 2–2.5 mm (%)	7.0	7.2	8.3	6.2	10.2	11.8	13.3	25.2	8.0	8.6	14.4	20.3	4.9	5.0	5.7	7.2
Grain fraction 2.5–2.8 mm (%)	30.5	34.6	33.4	24.6	39.6	37.3	44.9	55.6	32.3	32.9	32.4	39.2	27.0	26.9	28.4	32.5
Grain fraction >2.8 mm (%)	61.5	57.4	57.3	68.0	49.3	49.2	40.4	15.9	59.1	57.9	51.8	37.8	67.9	67.9	65.0	59.0
Thousand-grain weight (g)	39.3	38.9	37.2	37.9	39.7	37.9	36.6	29.4**	40.9	40.4	37.6	34.2*	44.8	44.2	42.4	40.6
Germination rate (%)	97.3	97.8	97.3	97.9	97.6	97.1	97.0	97.8	98.2	98.6	97.0	96.5	97.8	95.9	97.1	96.1
Total protein (%)	13.8	14.0	13.9	14.0	11.6	12.0	12.5	13.8	13.3	13.4	13.8	13.6	14.2	14.4	14.3	14.7
Wet gluten (%)	30.8	30.8	30.7	29.8	25.5	26.6	28.7	30.0	28.0	28.2	30.3	29.7	30.4	30.7	31.5	32.3
Hagberg falling number (s)	346.5	383.0	373.9	332.5	323.7	332.8	339.7	380.0*	333.1	326.9	362.9	376.8	331.3	379.5	359.9	370.0
Sedimentation value (mL)	40.9	38.4	36.5	39.6	38.1	39.9	43.2	46.1*	37.1	38.4	42.3	39.0	41.2	44.1	44.5	49.0*

*, **Indicate statistically significant differences, according to one-way ANOVA, between healthy and diseased plants at $P \leq 0.05$ and $P \leq 0.001$, respectively

Table 4 Summary of effects of sharp eyespot severity category (0–3), location (Ch, Mi, Mo, So) and year (2006, 2007, 2008, 2009) on grain yield and quality

Variate	Severity category	Location	Year	Interactions	
				Severity × Location	Severity × Year
Number of grains per ear	0^a 1^b 2^c 3^c	Ch^a Mi^b So^c Mo^d	07^a 09^a 06^b 08^c	ns	ns
Plant dry weight	0^a 1^b 2^c 3^d	Ch^a Mi^b So^b Mo^c	09^a 07^a 08^b 06^c	P=0.0017	P=0.0032
Ear dry weight	0^a 1^b 2^c 3^d	Ch^a Mi^b So^b Mo^c	09^a 07^b 08^c 06^c	P=0.0137	ns
Grain dry weight per ear	0^a 1^b 2^c 3^c	Ch^a Mi^b So^b Mo^c	09^a 07^b 08^{bc} 06^c	P=0.0124	ns
Thousand grain weight	0^a 1^a 2^b 3^b	So^a Ch^b Mo^b Mi^b	08^a 09^b 07^c 06^c	P=0.0075	ns
Germination rate	0^a 1^{ab} 2^{ab} 3^b	Mo^a Ch^{ab} So^b Mi^b	07^a 06^b 09^b 08^c	P=0.0037	P=0.0002
Protein content	–	So^a Ch^{ab} Mo^b Mi^c	–	ns	ns
Wet gluten content	0^a 1^{ab} 2^{ab} 3^b	So^a Ch^a Mo^b Mi^b	06^a 08^a 07^b 09^b	ns	ns
Hagberg falling number	0^a 1^{ab} 2^{ab} 3^b	–	06^a 08^{ab} 09^b 07^c	ns	ns
Sedimentation value	–	So^a Mi^{ab} Ch^b Mo^b	06^a 07^b 09^b 08^b	ns	ns

[a, b, c, d] Different letters indicate statistically significant differences, according to three-way ANOVA at $P \leq 0.05$

ns no significant interactions

There were no significant interactions of severity category × location on number of grains per ear, or of severity category × year on number of grains per ear, ear dry weight, grain dry weight per ear or thousand-grain weight. Percentage of grains in small fractions (<2 mm, 2–2.5 mm, 2.5–2.8 mm) often increased, and in larger fractions (>2.8 mm) decreased, with increased severity category. Sharp eyespot did not usually affect grain germination.

Increasing severity of sharp eyespot increased, but usually not significantly, protein and wet gluten contents in grain, and Hagberg falling number and sedimentation value (Table 3). Location significantly affected protein content, while severity category, location and year often significantly affected wet gluten content, Hagberg falling number and sedimentation value (Table 4).

Colonization of grain by fungi

Occurrence of sharp eyespot was associated with increased colonization of grain by fungi. On average there were 2.4–4.0 and 2.7–4.4 fungal isolates per grain from healthy and diseased plants, respectively, and 2.4–4.4 and 3.3–4.1 fungal isolates per grain from grain surface (non-disinfected) and interior (surface-disinfected), respectively (Table 5). *Alternaria alternata* and *Epicoccum nigrum* were the most common fungi. Both fungi occurred significantly more often on healthy plants only at Minikowo. *Arthrinium phaeospermum, Aspergillus niger, Botrytis cinerea, E. nigrum, Fusarium culmorum, Gibberella zeae*, *Khuskia oryzae, Microdochium bolleyi, Mucor mucedo, Penicillium granulatum* and *Trichoderma viride* occurred more often on the grain surface, and *A. alternata Cladosporium herbarum* and *Cochliobolus sativus* more often inside grain. *Cladosporium herbarum* and *Gibberella tricincta* tended to occur (but not significantly) more often in/on grain from healthy plants, and *Fusarium poae, K. oryzae, M. bolleyi* and *T. viride* on grain from diseased plants. The latter fungi seemed to be secondary colonizers which take advantage of the weakened tissue. *Fusarium langsethiae* occurred only inside grain. Its identity was confirmed by PCR amplification of DNA with SCAR primer, which produced the diagnostic 310-bp DNA fragment.

Discussion

A recent increase in sharp eyespot in cereals, including winter wheat, in Poland has been observed (Kurowski and Adamiak 2007; Lemańczyk 2010a, b). The situation in Poland resembles that in the Netherlands where, from 1974 to 1986, sharp eyespot increased in prevalence annually by an average of 0.4 % of culms and 4 % of fields affected (Daamen and Stol 1990). The disease has also become gradually more

Table 5 The most commonly occurring fungi on wheat grain from healthy stems and stems with sharp eyespot

Taxon	Non-disinfected grain (2007–2009)								Surface-disinfected grain (2006–2009)							
	Chrząstowo		Minikowo		Mochełek		Sobiejuchy		Chrząstowo		Minikowo		Mochełek		Sobiejuchy	
	H[a]	D[b]	H	D	H	D	H	D	H	D	H	D	H	D	H	D
	Percentage of total number of isolates															
Alternaria alternata (Fr.) Keissl.	45.5	44.3	33.1*	12.8*	44.7	36.7	36.6	44.6	65.2	57.8	36.0	46.7	53.6	56.0	57.5	57.8
Arthrinium phaeospermum (Corda) M.B. Ellis	0.2	0.5	0.8	0.4	1.3	0.2	1.0	0	0.3	1.1	0	0.6	0.3	0	0	0
Aspergillus niger Tiegh.	2.0	0.9	2.9	0.8	3.7	1.7	2.0	0.8	0	1.9	0	0.3	1.0	0.5	1.4	2.9
Botrytis cinerea Pers.	7.0	3.7	0.8	0.8	0.3	3.0	1.3	2.0	0	1.4	1.5	0.9	0.3	0.5	0.5	0.3
Cladosporium herbarum (Pers.) Link	0	0.5	0	0	0.3	0.5	0	0.3	5.0	3.1	14.6	11.1	4.6	8.8	3.3	0.3
Cochliobolus sativus (S. Ito & Kurib.) Drechsler ex Dastur	0	0	0	0	0.5	1.2	0.3	0	0	2.8	0	0	0.5	0.2	0.8	0.6
Epicoccum nigrum Link	23.4	20.5	37.6*	22.6*	25.9	25.1	16.8	22.1	17.3	14.7	22.0	18.1	21.9	15.9	16.3	16.3
Fusarium culmorum (W.G. Sm.) Sacc.	7.0	7.5	0	0.8	0	0	3.5	4.8	0.3	0.3	0	0	0	0	3.0	0.3
Fusarium langsethiae Torp & Nirenberg	0	0	0	0	0	0	0	0	0	0	1.2	0.9	0	0	0.3	0.3
Fusarium poae (Peck) Wollenw.	2.7	4.3	8.3	9.8	1.6	3.0	1.3	1.0	1.4	2.5	11.0	8.1	3.6	2.7	0.8	1.7
Gibberella avenacea R.J. Cook	1.2	0.5	0.8	0	0.5	1.2	1.0	1.8	1.7	0.3	0.9	2.7	1.0	0	2.2	0.9
Gibberella tricincta El-Gholl, McRitchie, Schoult. & Ridings	0	0.2	7.0	5.3	1.1	1.0	0	1.0	0	0.3	8.9	5.1	0.3	0.2	0	0.3
Gibberella zeae (Schwein.) Petch	1.7	0.9	2.1	0.4	0.5	0	1.8	0.8	1.9	1.1	0	1.2	0	0	1.6	0
Khuskia oryzae H.J. Huds.	0.5	0	2.1	0.8	1.9	7.4	2.5	6.8	0.6	0	0.3	0.6	3.9	4.2	4.4	4.7
Melanospora damnosa (Sacc.) Lindau	1.0	5.0	0.4	0	1.1	1.2	2.0	1.0	0.3	0	0	0.6	0.3	0	0.3	1.2
Microdochium bolleyi (R. Sprague) de Hoog & Herm.-Nijh.	4.5	6.8	0	0	7.0	9.4	7.0	10.0	2.2	5.0	0.6	0	7.5	5.9	3.8	5.5
Mucor mucedo Fresen.	0.5	0.5	0	0	1.3	0.7	0.3	0.3	0	0	0	0	0	0.2	0	0
Penicillium granulatum Bainier	0	0	0	38.5	0	0	19.3	0.5	0.3	0	0	0	0	0	2.7	0.3
Penicillium spp.	1.5	2.7	1.7	0	6.4	6.4	1.0	0.8	0.6	5.0	1.5	2.1	0	2.9	0	5.2
Trichoderma viride Pers.	0	0	0	6.8	0.5	0	1.5	0.8	0	0.8	0	0.3	0	0.2	0	0
Non-sporulating mycelia	0.2	0.5	0.4	0	0.8	0.7	0.5	0	1.7	1.7	0.3	0.3	0.8	0	0.8	0.3
Number of isolates per grain	4.0	4.4	2.4	2.7	3.7	4.1	4.0	4.0	3.6	3.6	3.4	3.3	3.9	4.1	3.7	3.4

[a] Grain from healthy shoots

[b] Grain from diseased shoots

*Indicate statistically significant differences, according to χ^2 – tests, between healthy and diseased plants at $P=0.05$

Fungi with frequency <1 %: on grain from healthy plants: *Aspergillus fumigatus* Fresen., *Gibberella intricans* Wollenw. and on grain from diseased stems and healthy plants: *Chaetomium funicola* Cooke, *Fusarium sporotrichioides* Sherb., *Gelasinospora cerealis* Dowding, *Ulocladium consortiale* (Thüm.) E.G. Simmons, *Stemphylium* sp., on grain from diseased stems: *Drechslera triseptata* (Drechsler) Subram. & B.L. Jain, *Pyrenophora erythrospila* A.R. Paul

severe in wheat and grass cropping areas of China, Italy, Russia, Turkey, Ukraine and the United Kingdom (Rossi et al. 1995; Colbach et al. 1997; Etheridge et al. 2001; Kryuchkova 2000; Tunali et al. 2008; Zhalieva 2008; Chen et al. 2010). Although sharp eyespot is not currently a major yield-limiting disease of wheat, considerable crop losses are possible in environments conducive to the disease.

Most studies suggest that only moderate or severe sharp eyespot incidence reduces yield substantially. In the West Midlands of England reduction in yield was only 5 % with 10–25 % incidence of disease and 20–25 % with 80 % incidence (Croxall et al. 1964). Severe infections in England and Wales reduced yields by an average of 26 % (Clarkson and Cook 1983), and in East Scotland by an average of 10–12 % (Pitt 1966). In New Zealand, yield loss was considered to be negligible when the incidence was <10 %, and maximum individual yield loss was estimated at 18 % (Cromey et al. 2002). Richardson et al. (1976) observed, however, that average reductions of 8 % in tiller yield caused a 75 % reduction in grain number and 25 % reduction in grain weight. Yield losses of more than 5 % are generally likely to occur in up to 20 % of crops.

In Poland, in 2006–2009, in three out of four sampled sites the greatest number of shoots with sharp eyespot had symptoms in the slight (1) category. Incidence of severe disease (category 3) was on average 3–10 times less than incidence of slight disease. An increase in average number of shoots with slight (category 1) disease was associated with decreased grain yield only at three sites. At Sobiejuchy, the high proportion of shoots in disease categories 1–3 was associated with relatively high grain yield, suggesting that conditions here were more suitable both for disease development and crop growth.

The disease appeared on stems soon after stem extension (GS 31 onwards), in May, and developed in the summer. The disease development differed from that in the United Kingdom where its decline in summer was observed by Nicholson et al. (2002). The summer development of disease in Poland was not masked by visible senescence symptoms (if compared with the healthy plants).

Even where slight disease predominated, most grain yield variates were affected, often significantly. The largest effect was in decreasing ear dry weight, a consequence of decreased number of grains per ear.

At Minikowo, Mochełek and Sobiejuchy, 10–25 % of the yield loss in severely infected shoots resulted from decreased grain number (and consequently grain dry weight per ear) and an additional 10–26 % from reduced grain weight. This may be a consequence of the timing of disease development; infection in stems that became severely diseased would be expected to have reached a critical level earlier than in those that became moderately diseased, affecting plant development between ear emergence and flowering and to a greater extent during grain filling. The lower grain number in severely infected shoots may also result from the positioning of these shoots. They are often the youngest and smallest tillers, with small grain numbers even if healthy, because of their shorter growth period and apical dominance by the older tillers.

Although an effect of sharp eyespot on decreasing grain yield is evident, especially within individual sites, grain yield generally seemed to be determined by the amount of mineral fertilization applied. The lowest average grain yield at Mochełek (3.95 $t\,ha^{-1}$) was associated with the lowest amounts of nitrogen (80 $kg\,ha^{-1}$) and phosphorus fertilization (17 $kg\,ha^{-1}$every year), and the highest average grain yield at Sobiejuchy (8.08 $t\,ha^{-1}$) with additional potassium. The latter observation supports findings of Snyder and Mascagni (1998) and Sweeney et al. (2000), who recorded increased yield in winter wheat in response to increased potassium fertilization.

It was shown that there is much variation in incidence of sharp eyespot resulting from location and year. The relatively severe disease in 2007 was associated with the relatively warm and wet autumn and winter followed by warmer and wet spring and colder and wet summer. Warmer winters and moist springs, which favour infection and colonization by *R. cerealis*, usually increase incidence and severity of disease (Clarkson and Cook 1983; Wiese 1987; Polley and Thomas 1991; Colbach et al. 1997; Cromey et al. 2002). Regional differences in incidence of sharp eyespot, which occurs most often in cooler regions, were also reported from the United Kingdom (Polley and Thomas 1991) and Canada (Hall and Sutton 1998).

Variation could also result from crop sequence, differences in mineral fertilization and type of fungicide applied. Sobiejuchy and Mochełek, with

the greatest incidence of shoots in disease categories 1–3, were the only sites where closely related cereals (triticale, spring and winter wheat) were used as preceding or pre-preceding crops. Sharp eyespot usually occurs more often in continuously cropped cereals, including particularly wheat (Colbach et al. 1997; Żółtańska 2005). White mustard used at Mochełek as the pre-preceding crop seemed not to contribute to disease suppression, although volatiles released from breakdown of thioglycosides have shown activity against soil-borne pathogens (Larkin et al. 2011). Low nitrogen rates at Mochełek did not decrease disease incidence as reported by Colbach et al. (1997). Different fungicides strategies were applied at different sites. Alternative application of flusilazole + carbendazim, spiroxamine + tebuconazole + triadimenol, kresoxim-methyl + epoxiconazole, fenpropimorph, azoxystrobin, prothioconazole + fluoxastrobin at Chrząstowo seemed to decrease the incidence of disease (only 49 % of shoots with sharp eyespot). Azoxystrobin, reported to be active against sharp eyespot (Bateman et al. 2000), was used at three locations in Chrząstowo in 2008, Minikowo in 2006 and 2007 and Sobiejuchy in 2006 and 2007. Its application was usually associated with significant reduction of disease. Cyproconazole has some growth-regulating activity that can result in plant growth retardation (Köller 1987). This fungicide did not however affect the growth of plants at Mochełek. The dry weight of plants from Mochełek was similar to those from other sites. Variation in disease was not expected to have resulted from soil type, which was similar in all sites, being dry, light, non-compacted sandy or silty loam rather poor in organic matter. Such soil generally favours survival and colonization by *R. cerealis* (Herman 1992; Cromey et al. 2002). An effect of increasing severity category on lower plant dry weight may suggest an involvement of soil moisture not only in disease incidence but also in plant development.

Average maximum yield loss in sampled sites was estimated at 8–10 % in a crop where 50 % of stems were infected, 25 % of them in severity categories 2 and 3. This yield loss is 2.5 times smaller than that predicted from a disease index calculated from the average proportion of tillers with severity categories 2 and 3. Such a disease index can be easily calculated. It is, however, more useful for estimating overall losses than losses in specific crops which may vary between locations and years.

Sharp eyespot tended to improve some measures of grain quality. Compared with healthy shoots, grain from diseased shoots often produced flour with more protein and wet gluten content, less alpha amylase activity, and slower sedimentation. Increased protein and gluten content are an expected consequence of decreased grain size because the proportion of endosperm carbohydrate is decreased. Protein and gluten have long been considered the most important functional constituents of wheat grain, with profound modifying effects on many functional properties of flour. Low alpha amylase activity (indicated by relatively high Hagberg falling number) is required in most baking flours as the enzyme converts starch to sugar, preventing proper dough characteristics. Slower sedimentation, shown in the tests, indicates both higher gluten content and better gluten quality.

The presence of sharp eyespot increased the incidence of *F. poae, K. oryzae*, *M. bolleyi* and *T. viride* on wheat grain. The importance of *F. poae* is increasingly recognized. It is one of the causal agents of fusarium head blight of wheat and may contribute to loss of grain yield and reduced germination. It has been associated with human and animal toxicoses as its strains produce a large number of mycotoxins, including trichothecenes of types A and B, beauvericin and enniatins (Kulik and Jestoi 2009; Stenglein 2009). *Fusarium* spp. can digest proteins and starch, and infected kernels can generate technical problems in production of bread (Betchel et al. 1985). *Khuskia oryzae* can affect grain quality, and decrease its viability and germination (Wiewióra et al. 2009). *Microdochium bolleyi* can contribute to winter wheat crown rot but with no significant effect on grain yield (Kane et al. 1987). *Trichoderma viride* produces many secondary metabolites active in its antimicrobial activity (Sivasithamparam and Ghisalberti 1998), which may however be toxic to humans (Cole and Cox 1981; de Hoog et al. 2000).

We have shown that sharp eyespot can affect yield in conventionally grown wheat in Poland. There was generally no effective fungicidal control (Colbach et al. 1997) or evidence of cultivar resistance against *R. cerealis* (Wiese 1987). Recently, however, Vibrance, a proprietary seed treatment fungicide based on the new active ingredient sedaxane was launched (Crummett 2011). If chemical control is restricted, as in organic farming, recognition of factors involved in development of disease in the moderate central European climate is necessary. Results of studies on effects of different crop

management procedures on incidence of disease may help in developing optimum practices for non-chemical disease control and maximum wheat yields.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

Anonymous. (2006). USDA National Nutrient Database for Standard Reference. http://www.nal.usda.gov/fnic/foodcomp/search/.

Anonymous. (2010). World Wheat, Corn and Rice. Oklahoma State University, FAOSTAT. http://nue.okstate.edu/crop-information/world-wheat-production.htm.

Bateman, G. L., Edwards, S. G., Marshall, J., Morgan, L. W., Nicholson, P., Nuttall, M., Parry, D. W., Scrancher, M., & Turner, A. S. (2000). Effects of cultivar and fungicides on stem-base pathogens, determined by quantitative PCR, and on diseases and yield of wheat. *Annals of Applied Biology, 137*, 213–221.

Betchel, D. B., Kaleikan, L. A., Gaines, R. L., & Seitz, L. M. (1985). The effects of *Fusarium graminearum* infection on wheat kernels. *Cereal Chemistry, 62*, 191–197.

Carling, D. E., & Sumner, D. R. (1992). *Rhizoctonia*. In L. L. Singleton, J. D. Mihail, & C. M. Rush (Eds.), *Methods for research on soilborne phytopathogenic fungi*. St Paul: American Phytopathological Society Press.

Chakraborty, S., Tiedemann, A. V., & Tent, P. S. (2000). Climate change: potential impact on plant diseases. *Environmental Pollution, 108*, 317–326.

Chen, H. G., Cao, Q. G., Xiong, G. L., Li, W., Zhang, A. X., Yu, H. S., & Wang, J. S. (2010). Composition of wheat rhizosphere antagonistic bacteria and wheat sharp eyespot as affected by rice straw mulching. *Pedosphere, 20*, 505–514.

Clarkson, J. D. S., & Cook, R. J. (1983). Effects of sharp eyespot on yield loss in winter wheat. *Plant Pathology, 32*, 421–428.

Colbach, H., Lucas, P., Cavelier, N., & Cavelier, A. (1997). Influence of cropping system on sharp eyespot in winter wheat. *Crop Protection, 16*, 415–422.

Cole, R. J., & Cox, R. H. (1981). *Handbook of toxic metabolites* (pp. 152–263). New York: Academic.

Cromey, M. G., Butler, R. C., Boddington, H. J., & Moorhead, A. R. (2002). Effects of sharp eyespot on yield of wheat (*Triticum aestivum*) in New Zealand. *New Zealand Journal of Crop and Horticultural Science, 30*, 9–17.

Cromey, M. G., Butler, R. C., Munro, C. A., & Shorter, S. C. (2005). Susceptibility of New Zealand wheat cultivars to sharp eyespot. *New Zealand Plant Protection, 58*, 268–272.

Croxall, H. E., Dale, W. T., & Knight, B. C. (1964). The incidence of soil-borne diseases of cereals in the West Midlands 1959–63. *Proceedings of the British Insecticide and Fungicide Conference, 1963*, 223–231.

Crummett, D. (2011). New Vibrance fungicide stops rhizoctonia. *Crop Production*. www.FarmProgress.com.

Daamen, R. A., & Stol, W. (1990). Surveys of cereal diseases and pests in the Netherlands2. Stem-base diseases of winter wheat. *Netherland Journal of Plant Pathology, 96*, 251–260.

de Hoog, G. S., Guarro, J., Gené, J. & Figueras, M. J. (2000). *Atlas of Clinical Fungi* (2nd ed., p. 1126). Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures.

Etheridge, J. V., Davey, L., & Christian, D. G. (2001). First report of *Rhizoctonia cerealis* causing sharp eyespot in *Panicum virgatum* in the UK. *Plant Pathology, 50*, 807.

Hall, R., & Sutton, J. C. (1998). Relation of weather, crop, and soil variables to the prevalence, incidence, and severity of basal infections of winter wheat in Ontario. *Canadian Journal of Plant Pathology, 20*, 69–80.

Herman, M. (1992). The effect of soil moisture on the incidence of *Rhizoctonia cerealis* and microbial antagonism against it. II Antagonism of the soil against *R. cerealis* as affected by temperature and soil water content. *Scientia Agriculturae Bohemoslovaca, 24*, 317–325.

Huynh, H., & Feldt, L. S. (1970). Conditions under which mean square ratios in repeated measurements designs have exact *F*-distributions. *Journal of the American Statistical Association, 65*, 1582–1589.

Kane, R. T., Smiley, R. W., & Sorrells, M. E. (1987). Relative pathogenicity of selected *Fusarium* species and *Microdochium bolleyi* on winter wheat in New York. *Plant Disease, 71*, 177–181.

Köller, W. (1987). Isomers of sterol synthesis inhibitors: fungicidal effects and plant growth regulator activities. *Pesticide Science, 18*, 129–147.

Kryuchkova, L. (2000). Stem-base diseases of wheat in Ukraine. *Proceedings of the BCPC Conference—Pests & Diseases, 2000*, 113–118.

Kulik, T., & Jestoi, M. (2009). Quantification of *Fusarium poae* DNA and associated mycotoxins in asymptomatically contaminated wheat. *International Journal of Food Microbiology, 130*, 233–237.

Kurowski, T. P., & Adamiak, E. (2007). Occurrence of stem base diseases of four cereal species grown in long-term monocultures. *Polish Journal of Natural Sciences, 22*, 574–583.

Larkin, R. P., Honeycutt, C. W., & Olanya, O. M. (2011). Management of *Verticillium* wilt of potato with disease-suppressive green manures and as affected by previous cropping history. *Plant Disease, 95*, 568–576.

Lemańczyk, G. (2010a). Occurrence of sharp eyespot in spring cereals grown in some regions of Poland. *Journal of Plant Protection Research, 50*, 505–512.

Lemańczyk, G. (2010b). Occurrence of sharp eyespot (*Rhizoctonia cerealis*) in winter triticale grown in some provinces of Poland. *Phytopathologia, 56*, 27–38.

Maxwell, S. E., & Delaney, H. D. (1990). *Designing experiments and analyzing data: a model comparison perspective*. Belmont: Wadsworth Publishing Company.

Mikolajska, J. & Wachowska, U. (1996). *Characterization of binucleate isolates of Rhizoctonia cerealis with respect to cereals*. In M. Kowalik, & S. kowalski (eds.), *North*

Eastern Poland Symposium on New Directions in Plant Pathology, Sep 11–13 (pp. 303–306) Kraków, Poland.

Nicholson, P., & Parry, D. W. (1996). Development and use of a PCR assay to detect *Rhizoctonia cerealis*, the cause of sharp eyespot in wheat. *Plant Pathology, 45*, 872–883.

Nicholson, P., Turner, A. S., Edwards, S. G., Bateman, G. L., Morgan, L. W., Parry, D. W., Marshall, J., & Nuttall, M. (2002). Development of stem-base pathogens on different cultivars of winter wheat determined by quantitative PCR. *European Journal of Plant Pathology, 108*, 163–177.

Nyvall, R. F. (1989). *Field crop diseases handbook* (2nd ed.). New York: Van Nostrand Reinhold.

Pitt, D. (1964). Studies on sharp eyespot disease of cereals. I. Disease symptoms and pathogenicity of isolates of *Rhizoctonia solani* Kühn and the influence of soil factors and temperature on disease development. *Annals of Applied Biology, 54*, 77–89.

Pitt, D. (1966). Studies on sharp eyespot disease of cereals. III. Effects of the disease on the wheat host and the incidence of disease in the field. *Annals of Applied Biology, 58*, 299–308.

Polley, R. W., & Thomas, M. R. (1991). Surveys of diseases of winter wheat in England and Wales, 1976–1988. *Annals of Applied Biology, 119*, 1–20.

Prange, A., Birzele, B., Krämer, J., Meier, A., Modrow, H., & Köhler, P. (2005). *Fusarium*-inoculated wheat: deoxynivalenol contents and baking quality in relation time. *Food Control, 16*, 739–745.

Prew, R. D., & McIntosh, A. H. (1975). Effects of benomyl and other fungicides on take-all, eyespot and sharp eyespot diseases of winter wheat. *Plant Pathology, 24*, 67–71.

Richardson, M. J., Whittle, A. M., & Jacks, M. (1976). Yield loss relationships in cereals. *Plant Pathology, 25*, 21–30.

Rossi, V., Cervi, C., Chiusa, G., & Languasco, L. (1995). Fungi associated with foot rots on winter wheat in northwest Italy. *Journal of Phytopathology, 143*, 115–119.

Siuda, R., Grabowski, A., Lenc, L., Ralcewicz, M., & Spychaj-Fabisiak, E. (2010). Influence of the degree of fusariosis on technological traits of wheat grain. *International Journal of Food Science and Technology, 45*, 2596–2604.

Sivasithamparam, K., & Ghisalberti, E. L. (1998). Secondary metabolism in *Trichoderma* and *Gliocladium*. In C. P. Kubicek & G. E. Harman (Eds.), *Trichoderma & Gliocladium* (pp. 139–191). London: Taylor & Francis.

Snyder, C. S. & Mascagni, H. J. (1998). Phosphorus and potassium increase wheat yields and help reduce disease damage. *News and Views from the Southeast Region,* Int. Plant Nutrition Institute. October 1998. http://oilpalm.ipni.net/ppiweb/ppinews.nsf/0/a08e675e4e0505e58525691b006678f2/$FILE/98166-CSS-Wheat%20Yields.pdf. Accessed 25 October 2011.

Stenglein, S. A. (2009). *Fusarium poae*: a pathogen that needs more attention. *Journal of Plant Pathology, 91*, 25–36.

Sweeney, D. W., Granade, G. V., Eversmeyer, M. G., & Whitney, D. A. (2000). Phosphorus, potassium, chloride, and fungicide effects on wheat yield and leaf rust severity. *Journal of Plant Nutrition, 23*, 1267–1281.

Tunali, B., Nicol, J. M., Hodson, D., Uçkun, Z., Büyük, O., Erdurmuş, D., et al. (2008). Root and crown rot fungi associated with spring, facultative, and winter wheat in Turkey. *Plant Disease, 92*, 1299–1306.

Van Der Hoeven, E. P., & Bollen, G. J. (1980). Effect of benomyl on soil fungi associated with rye. 1. Effect on the incidence of sharp eyespot caused by *Rhizoctonia cerealis*. *Netherlands Journal of Plant Pathology, 86*, 163–180.

Wiese, M. V. (1987). *Compendium of wheat diseases* (2nd ed.). St Paul: APS Press.

Wiewióra, B., Mańkowski, D., & Bulińska-Radomska, Z. (2009). Zdrowotność ziarna zbóż pochodzącego z ekologicznej produkcji nasiennej (Health of cereals seed originated from ecological seed production). *Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 251*, 29–39.

Willis, W. G. (1984). *Wheat diseases*. Manhattan: Cooperative Extension Service, Kansas State University. Publication S–23.

Wilson, A., Simpson, D., Chandler, E., Jennings, P., & Nicholson, P. (2004). Development of PCR assays for the detection and differentiation of *Fusarium sporotrichioides* and *Fusarium langsethiae*. *FEMS Microbiology Letters, 233*, 69–76.

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research, 14*, 415–421.

Zhalieva, L. D. (2008). Changes in a pathogenic complex of major pathogens causing root rot on grain cereals in conditions of Krasnodar. In Territory abstract. *International Conference on Information Systems of Diagnostics, Monitoring and Forecasting the Major Weed Plants, Pests and Diseases of Agricultural Crops,* May 12–16 (pp. 105–106) St. Petersburg-Pushkin, Russia, 120 pp.

Żółtańska, E. (2005). The effect of previous crop and weather conditions on the incidence of stem base diseases in winter wheat. *Journal of Plant Protection, 45*, 37–40.